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Resumen por el autor, Alfred S. Romer.

La comparación de los coracoides de los mamíferos y reptiles.

Para establecer las homologías de los elementos del coracoides de los mamíferos con los del de los reptiles, debe compararse la cintura escapular de los monotremas con la de los antepasados terápsidos de los mamíferos, y no con la de los reptiles modernos que no están relacionados con ellos. Los argumentos de Gregory y Hanson para demostrar que el coracoides y epicoracoides de los monotremas son homólogos con el 'coracoides' y 'epicoracoides,' respectivamente, de los reptiles vivientes, no son suficientes para demostrar este punto.

Cuando se consideran los coracoides de los mamíferos y reptiles en su verdadero orden filético, conforme han hecho Broom y Watson, puede verse que el coracoides de los mamíferos es el elemento primitivo posterior, que falta en los reptiles modernos. El 'coracoides' de estos últimos es el elemento primitivo anterior, homólogo al epicoracoides de los monotremas, faltando en los mamíferos superiores.

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THE COMPARISON OF MAMMALIAN AND REPTILIAN CORACOIDS

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ONE FIGURE

The problem of the homologies of mammalian and modern reptilian coracoids has been complicated by the palaeontological discoveries of the past twenty years, especially those of Broom, Case, Watson, and Williston which show that most of the extinct primitive reptiles and all of the extinct mammal-like forms (therapsids) possessed two coracoid elements.

Williston, in 1911, demonstrated that the modern reptilian 'coracoid' corresponds to the anterior of the two primitive elements, and this interpretation is generally accepted. No agreement, however, has been reached on the question of the derivation of the mammalian coracoid.

In 1912 Broom compared the mammalian coracoid with that of the therapsid ancestors of the mammals, and concluded that it corresponds to the posterior coracoid of the more primitive forms and is not homologous, therefore, with the 'coracoid' of existing reptiles. In 1917 Watson traced the two primitive coracoids through a graded phyletic series of mammal-like reptiles to the two coracoid ossifications of the monotreme, and confirmed Broom's results. Gregory, on the other hand, compared the monotreme girdle with that of lizards and *Sphenodon*, and in 1918 reached the opposite conclusion, namely, that the coracoid of mammals corresponds to that of living reptiles—the anterior primitive element. He suggested also that the epicoracoid bone of monotremes may correspond to the 'epicoracoid' cartilage of lizards and *Sphenodon* and to a similar cartilage which is generally believed to have existed in a num-

ber of extinct reptiles. He notes that Watson's paper was received too late for consideration.

In 1920 Hanson restated Gregory's argument, without adding any new element to the discussion. Additional citations were made to show the probable presence of an 'epicoracoid' cartilage in extinct forms. The monotreme girdle was again compared with that of the lizards and *Sphenodon*. Watson's work was mentioned, but not considered, and, although three years had elapsed, no comparison was made between the girdle of the monotreme and that of its therapsid ancestors.

All are agreed as to the homologies of the coracoids within the limits of the class Reptilia. A few of the most primitive Permian forms (as *Seymouria*) have but one element, the anterior (fig. 1). Most other primitive fossil reptiles have in addition a second element which carries the posterior half of the glenoid cavity. From these primitive types the modern reptilian coracoid is derived, either through the secondary loss of the posterior element or through the retention of the most primitive condition of the girdle in which the posterior coracoid had not yet made its appearance. The mammal-like reptiles, on the other hand, possess two coracoids which are clearly homologous with those of their primitive ancestors. In this type of girdle the anterior element is almost completely excluded from the glenoid cavity by the posterior one (Watson).

Within the class Mammalia, the homologies are unquestioned also. The coracoid of the adult marsupial is clearly that of the placental. In the embryo, as noted by Broom ('97, '99, '02, '12) and confirmed by Watson ('17) and by Hanson ('20), it extends from the glenoid cavity to the midline. The condition of the coracoid of the embryonic marsupial is repeated in the coracoid of the adult monotreme. The epicoracoid of the latter is represented, according to Broom, by a membrane anterior to the coracoid of the embryonic marsupial.

It remains, then, to compare the two classes. It is in the selection of the proper forms between which to make the comparison, that the workers belonging to the two groups disagree. The monotreme is taken by all as representing the most primi-

tive mammalian condition. Broom and Watson compare the monotreme with the therapsids, from which it is generally agreed that the mammals have been derived. The therapsids possess two coracoid elements, of which the posterior one occupies the lower half of the glenoid cavity and extends downward and backward from it. This is the position occupied by the true coracoid of monotremes. Anteriorly, in the therapsid, is the primitive anterior coracoid element, and the epicoracoid is anteriorly placed in the monotreme. The anterior coracoid of the therapsid and the epicoracoid of the monotreme occupy essentially the same position, and both are excluded from the glenoid. The only noticeable difference lies in the appearance of a gap in the upper edge of the monotreme epicoracoid, which does not reach the scapula above as does the therapsid anterior coracoid. The appearance of this gap has been explained by Watson upon functional grounds.

Both Gregory and Hanson compare the coracoid of the monotreme with that of *Sphenodon* and the lizards. Hanson states that 'genetic connection and homology' exist between them. This 'genetic connection,' of course, would not apply to any phyletic relationship between the groups of animals involved. There is no evidence, other than the suggested homology of the coracoids, which would tend to show that either *Sphenodon* or the lizards, which belong to two widely divergent subclasses of reptiles, are in any way ancestral to mammals. On the other hand, almost all students of mammalian phylogeny, such as Owen, Seeley, Broom, Gregory, and Watson, are agreed that the therapsids do occupy such a position.

The two modern reptilian groups mentioned and the ancestors of the mammals have followed separate lines of development since the Permian at least. Admittedly the shoulder-girdle of both the modern reptile and the monotreme has been greatly modified from the common primitive type. It is difficult to understand how a 'genetic connection' is possible between the shoulder-girdles of what appear to be end forms of divergent lines. The common meeting-point for the establishment of a genetic connection between mammals and modern reptiles is

the primitive reptile of the Permian, and it is generally accepted that this common ground is to be reached from the mammalian side only through the therapsids.

Three main lines of argument are followed by Gregory and Hanson in making the comparison: 1) The epicoracoid of monotremes is held to be the homologue of the 'epicoracoid' cartilage of lizards and *Sphenodon* and of a cartilaginous area which existed anterior to the anterior coracoid of some primitive and mammal-like reptiles. 2) The muscular origins on the two coracoid bones of monotremes are similar to those on the coracoid and 'epicoracoid' of lizards and *Sphenodon*. 3) The position of the epicoracoid and coracoid of monotremes is similar to that of 'epicoracoid' cartilage and coracoid in lizards and *Sphenodon*. These arguments may now be examined.

1. The epicoracoid: In monotremes this is an independent ossified element. The so-called epicoracoid of lizards and *Sphenodon* is a cartilage which, as the development of the shoulder-girdle of the lizard would tend to show, is merely a peripheral portion of the primary girdle into which ossification has not extended. It is found not only anterior, but ventral to the 'coracoid.' Cartilage is assumed to have occupied both these positions in many of the extinct primitive and mammal-like reptiles. This cartilage seems to have been somewhat variable in its development; some well-preserved Permian girdles show little possibility of cartilage anteriorly. As Hanson admits, there is no evidence that a bone was ever present in this position.

This cartilage Gregory and Hanson compare with the epicoracoid bone in the monotreme. But the monotreme also preserves an anterior and ventral peripheral cartilage which borders the epicoracoid bone exactly as the 'epicoracoid' cartilage of reptiles borders the anterior coracoid element (Parker, '68, p. 192). The value of any comparison that may be drawn between a variable peripheral reptilian cartilage and the distinct and ossified epicoracoid of the monotreme would seem to be open to serious question, especially in view of the fact that the cartilage itself is present in the latter form.

2. The muscular relations of the elements: Two objections may be raised to a comparison of the muscular origins of the coracoid plate of lizards and *Sphenodon* with those of the coracoid elements of the monotreme. If the single coracoid of modern reptiles replaces functionally the two primitive elements of the coracoid plate, the musculature borne by the single modern reptilian bone would have been distributed between two elements in the primitive forms and in the ancestors of the mammals. Further, if, in the evolution of the mammals, a portion of the musculature of the coracoid plate has moved upward on to the scapula, as it is generally believed to have done, marked divergence in the monotreme from the primitive condition of the musculature of the coracoid elements would have been expected.

Gregory compares the muscular relations of the 'coracoid' of *Sphenodon* with those of the coracoid of the monotremes, and states quite correctly that the coracobrachialis and biceps arise from the supposedly homologous bones in either case. But differences are also found. In the monotreme the epicoracoid shares in the origin of the biceps. Further, the *Sphenodon* 'coracoid' bears a large portion of the origin of the supracoracoideus. This muscle is the homologue of three muscles in the monotreme, the supraspinatus, infraspinatus, and supracoracoideus proper.¹ These derivatives are found upon the epicoracoid and scapula of the latter form. Hence the musculature of the *Sphenodon* 'coracoid' is distributed between all three elements of the primary shoulder-girdle of the monotreme.

The musculature of the 'epicoracoid' cartilage of *Sphenodon* is compared with that of the monotreme epicoracoid by Gregory. As he states, this cartilage in *Sphenodon* bears a portion of the origin of the supracoracoideus; it also gives origin to part of the scapulohumeralis anterior. In the monotreme the supracoracoideus proper originates from the epicoracoid bone, but the supraspinatus and infraspinatus, which are probably also derived from the reptilian supracoracoideus, are associated not with this bone, but with the scapula. The *teres minor*, the

¹ This matter is discussed more fully in a paper shortly to appear in the Bulletin of the American Museum of Natural History, 1922.

probable homologue of the reptilian scapulohumeralis anterior, does not arise from the epicoracoid, but from the scapula. Thus the musculature attached to the reptilian 'epicoracoid' cartilage is found in the monotreme to be attached for the most part to the scapula.

The muscular relationships, then, can scarcely be said to prove the homologies that have been suggested. It is of interest to note that Watson has used these relationships, by the seemingly more legitimate comparison of the monotreme muscles with their probable distribution in the mammal-like reptiles, in arriving at an entirely different conclusion.

3. The argument from the position: Gregory is followed by Hanson in comparing the spatial relations of the elements of the monotreme with those of the modern reptilian shoulder-girdle. The questionable value of this comparison has been referred to on page 42.

The so-called epicoracoid of lizards and *Sphenodon* is widely excluded from the glenoid, and the same is said by Gregory and Hanson to be true also of the epicoracoid of the monotreme. Although the latter element is excluded from the glenoid, it can scarcely be said to be 'widely' excluded, if viewed from the outer aspect. If this argument of exclusion from the glenoid be applied to the therapsids, it will be found to apply to the anterior coracoid element (Watson). This agrees with Broom's and Watson's homology of this element with the monotreme epicoracoid.

In modern reptiles the glenoid is bordered by the surviving anterior coracoid element. But in the higher therapsids the glenoid cavity is bordered by the posterior coracoid. This is also the case with the true coracoid of monotremes, which again bears out the conclusion of Broom and Watson.

The position of the two bony elements of therapsids is almost identical with that of the two bony elements of the monotremes. The primitive posterior coracoid of the therapsid occupies the same position as that of the true coracoid of the monotreme. The primitive anterior element occupies essentially the same position as that of the monotreme epicoracoid. To make a

comparison between these two related groups under the theory of Gregory and Hanson, one must imagine that, 1) while the anterior 'epicoracoid' cartilage was retained, an ossified epicoracoid made its appearance behind it and took up the position of the anterior coracoid of therapsid. Also, 2) that the anterior coracoid moved into the exact position of the posterior coracoid of therapsids, and, 3) the posterior coracoid of the mammal-like reptiles, which had grown constantly in size throughout the therapsid series, was replaced in all its relations by the anterior element, and disappeared completely.

If, however, one concludes with Broom and Watson that the mammalian coracoid is the primitive posterior element, the practical identity of position between the two elements of monotremes and the two bony elements of the mammal-like reptiles is quite evident.

Hanson says: "It may be pertinent to ask, if the coracoid process of placental mammals is the posterior element of Permians, how did it get to the anterior side of the glenoid? It is hard to imagine any rotation or migration of this element which would bring it from a position distinctly posterior of the glenoid to its present distinctly anterior position." It may be noted in this regard that the Permian posterior coracoid is never 'distinctly posterior of the glenoid.' It carries half the glenoid surface, while the therapsid posterior coracoid occupies completely, or almost completely, the ventral half of the glenoid. The mammalian coracoid is not always 'distinctly anterior' to the glenoid. In the monotreme it occupies the ventral half of the glenoid, as does the therapsid posterior coracoid. The two elements seem clearly homologous; we can trace nearly every gradation between the placental coracoid process and the posterior coracoid of the primitive Permian reptiles. Further, since the modern reptilian 'coracoid' is the anterior element, it is not the homologue of the true mammalian coracoid, but of the epicoracoid of monotremes.

SUMMARY

1. The monotreme shoulder-girdle should be compared with that of the forms from which the mammals are believed to have been derived (therapsids), and not with that of unrelated modern reptiles.

2. The mammalian coracoid is the posterior coracoid element of primitive and mammal-like reptiles, as stated by Broom and Watson.

3. The modern reptilian 'coracoid' is homologous with the primitive anterior element and the epicoracoid of monotremes.

4. The so-called epicoracoid of modern reptiles is a cartilage which may be compared with a variable anterior and ventral cartilage in extinct reptiles and a similarly placed cartilage in monotremes.

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Resumen por el autor, Walter E. Sullivan.

La función de los discos articulares.

Los discos articulares se encuentran bajo una forma simple y diagramática en el cráneo del bacalao, *Gadus morrhua*. Relacionando estos discos con los hallados en el hombre, el autor ha encontrado que los del último pueden reducirse á simples formas geométricas, las cuales indican que en todos los casos compensan las desigualdades de las superficies opuestas con el fin de mantener la estabilidad, careciendo de otra función.

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THE FUNCTION OF ARTICULAR DISCS

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TWO FIGURES

The present work is incidental to a study of the skull of the cod, *Gadus morrhua*, by dissection rather than by maceration. While making this dissection several examples of complete articular discs were found, and these seemed to clarify to some extent the views held in regard to the function of the discs.

Several general views are held in regard to the function of the discs. These are well reviewed by Fick and need be referred to only briefly here. The one most generally held is that they compensate for incongruities of the opposed surfaces. It has been held that they represent vestigial skeletal elements; that they make the lubrication of the joints more simple; that they are shock absorbers, and that they increase the range of movement of the joint. The elementary fact that in the structure of joints only two factors, mobility and stability, may be considered is frequently lost sight of.

It may be pointed out that relatively little work has been done on the joints in fishes. Petersen's work is a marked exception. In some ways the skull of the cod is more complex than the skull of the vertebrates grouped above it and diarthrodial joints exist between skeletal elements which in higher forms are united through a synarthrosis. The maxilla and premaxilla may be cited as examples.

The discs in man are frequently irregular, vary in a slight degree in different individuals, and in this way have their primary function somewhat obscured. In the cod, on the contrary, they are relatively simple and diagrammatic in outline, and but little doubt is left in regard to their function. Here an attempt will

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be made to correlate the discs found in the cod with those found in man, by reducing them to simple geometrical figures.

Two general types of discs are found in the cod, both of which are biconcave and elliptical in the outline of the margins. In one type the long axes of the concavities are parallel to each other; in the other type the long axes of the concavities are at right angles to each other.

In man the discs occur in various shapes. There is the biconcave type of the temporomandibular joint; the irregular but usually concavoconvex disc of the sternoclavicular joint; the cuneiform disc of the wrist-joint. The menisci of the knee-joint are in pairs, so that the result is that of a biconcave or planoconcave disc.

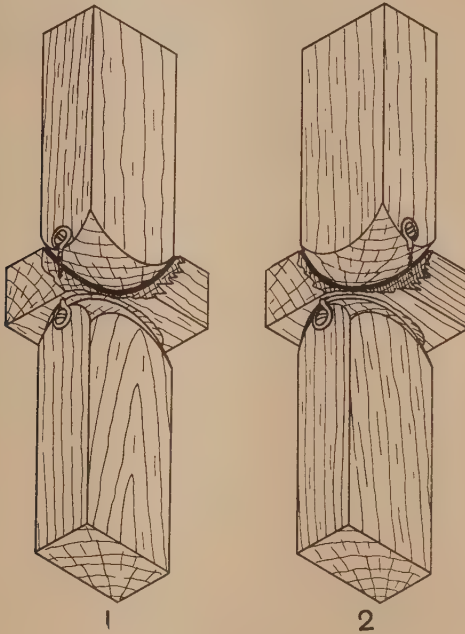
In the formation of joints there may be all types of shapes in the articular surfaces. The greatest degree of mobility and the smallest degree of stability would be attained by opposing two convex surfaces, preferably segments of spheres, to each other. The almost complete absence of stability precludes the use of this arrangement. In the shoulder-joint we have an approach to the opposition of a plane and a convex surface. The shallow glenoid cavity and the weakly developed labrum add but little to the stability of the joint. In the hip-joint, on the contrary, there is a well-balanced condition of mobility and stability.

By the addition of an articular disc, stable joints may be established between surfaces that would otherwise lack stability. First, by way of illustration, the condition of a diarthrosis in the midline may be considered. The opposing surfaces of necessity will be symmetrical. If two plane surfaces are opposed there is stability but little mobility. If the opposing surfaces are convex, the reverse is true. If both mobility and stability are desirable, it is practically reduced to a condition of two convex articular surfaces with a biconcave disc interposed.

The presence of a biconcave disc with the long axes of the concavities parallel may be considered. Figure 1 illustrates this type. In man the temporomandibular joint is the nearest approach to it. In general it cannot be held that this type holds any very decided advantage over the condyloid joint. The range

of movement is usually slightly greater without any loss in stability and a small amount of voluntary rotation is usually added to the movements permitted in the condyloid type.

Then there is the type where the disc is biconcave or concavo-convex, but with the long axis of one articular surface at right angles, or nearly so, to the long axis of the other articular surface.



Figs. 1 and 2

This is met with in a simple form in the joint between the maxilla and the premaxilla in the cod and in a modified form in the sternoclavicular joint in man. This gives a very stable type of joint which is essentially biaxial, but which usually permits a small amount of rotation. Figure 2 illustrates this type. Movements in one plane may take place by one of the bones' moving on the disc, and movements in the other plane would then take place by the same bone and the disc moving on the

other bone. If the movements are unrestricted, as is the case in fishes, either bone may move on the disc, or the disc and either of the bones may move on the other bone. In all of these movements the areas of contact are at a maximum and a large degree of stability is assured.

It may be pointed out that the long axes of the disc of the sternoclavicular joint are not usually at right angles to each other; but in general it can be correlated with the principles just stated. On the basis of the movements permitted, it seems that this joint should be grouped with the enarthrodial joints.

The articular disc at the wrist-joint is in many ways the most interesting of the accessory joint structures. Its shape is changed but little during the ordinary range of pronation and supination, and so the shapes of the opposed surfaces at the wrist-joint remain about constant and its stability is not impaired during the excursion of the radius upon the ulna.

SUMMARY

It seems hardly necessary to discuss the discs as shock absorbers. The presence of joints in general may be considered as a factor in the absorption of shock, but the articular discs are not placed in positions that would indicate that they play any important part. Indirect evidence is offered by the fact that among the bones most frequently fractured are those associated with articular discs.

Lubrication is a function of the synovial membrane. Opinion seems about equally divided as to the presence of synovial membrane over the articular discs. My personal conviction is that it is present, for at the wrist-joint I have succeeded in isolating it as a gross structure by dissection. Increased lubrication, however, is effected most simply by folds of the synovial membrane. The discs present unbroken surfaces.

It is hardly proper to refer to them as vestigial skeletal elements, for they are themselves skeletal elements and are found in vertebrates as low as the fishes, and here, indeed, in their greatest perfection.

Freedom of movement depends on the shape of the articular surfaces and upon the length of the capsule and accessory ligaments. In some cases the adjacent muscles may play a part. The discs in themselves do not increase the range of movement, but only contribute to the stability of the joints during increased movement dependent on other factors.

The articular discs may be reduced to geometrical forms which compensate for the incongruities of the opposed surfaces to the end of maintaining stability. They should be considered stabilizers only. The other functions attributed to them either do not exist or are incidental to their primary function. My own conclusion is that the other functions do not exist.

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The two drawings for figures 1 and 2 of this paper were made by W. A. Werrel, to whom I wish to express my thanks.

Resumen por los autores, P. N. Johnstone, F. H. Wakefield y
H. M. Currey.

Sobre la vascularidad relativa del músculo cardíaco y de las
fibras de Purkinje.

En la presente investigación se ha comparado la vascularidad de las fibras de Purkinje y del músculo cardíaco del corazón de la ternera. Los autores han encontrado que las fibras de Purkinje están mucho menos vascularizadas que el músculo cardíaco adyacente. El haz principal es un poco más vascular que los miembros. En el haz de His los vasos precapilares están confinados, en general, al tejido situado entre las fibras de Purkinje mientras que los capilares están dispuestos en su periferia. En el haz principal y en las porciones superiores de los miembros los vasos sanguíneos no penetran dentro de las fibras de Purkinje. En las porciones inferiores de los miembros y en las bandas moderadoras las fibras están atravesadas por vasos sanguíneos. A causa de la diferencia de la vascularidad entre el músculo cardíaco y las fibras de Purkinje, los autores creen que estas no representan probablemente un tejido especializado para el mismo tipo de contractilidad del músculo cardíaco.

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ON THE COMPARATIVE VASCULARITY OF HEART MUSCLE AND OF THE PURKINJE FIBERS

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TWO FIGURES

Curran, in describing a constant bursa in relation to the Bundle of His, states: "The existence of the bursa tends to prove that either the auriculo-ventricular bundle does not contract at all, or that it contracts in a different way and at a different time from the contraction of the ventricles." Erlanger, in studying the physiology of Purkinje tissue, observed, after numerous trials, a case in which there seemed to be independent movement of false tendons. His work was done on the calf's heart. Regarding the contractility, he says: "Owing to the difficulty of reaching the false tendons and to the fact that they move with the part of the heart to which they remain attached, it was found impossible to record satisfactorily their movements. Throughout the research, however, the false tendons have been closely observed for the purpose of determining whether they contract. The cut tendon lying against the smooth, moist endocardium, or hanging freely into the cavity of the ventricle would certainly have moved perceptibly had any of its elements contracted. In only one case did the false tendons seem to move independently of the heart wall. In this case the false tendons contained no heart muscle."

If the Purkinje fibers represent tissue specialized for the same type of contractility as heart muscle, one would expect them to have a vascular supply equal in abundance to that of heart muscle. We have, in this study, compared the vascularity of the Purkinje fibers and of heart muscle.

The accompanying illustrations are from drawings made with the camera lucida. The sections were made from calves' hearts, the coronary arteries of which had been injected with India ink. The hearts were injected at the slaughter-house immediately after the animals had been killed, and in all cases were soft and pliable; that is, they were not yet in a state of contracture. In most cases the auricles were fibrillating. The injections were all done with a syringe, only a medium pressure being used. If the heart was injected before it was in a state of contracture, it was not found necessary to use high pressure in order to obtain a capillary injection.

The first step in the injection was to split the right posterior portion of the aorta longitudinally. Care was taken to split only the aortic wall. By spreading the cut surfaces of the aorta, the sinuses of Valsalva were exposed. The left posterior sinus was then circled with a sharp knife from the inside of the aortic wall. The sinus was then freed from the underlying connective tissue, and the cannula was inserted, the tie being placed around the aortic border of the sinus. This procedure was duplicated on the anterior sinus. In this manner every branch of either coronary artery was injected, and at the same time the ink was, for the most part, kept out of the left ventricle. The heart was then immediately opened, the larger blood vessels being clamped with hemostats, and the His bundle was dissected out. Seepage of ink from the smaller blood vessels was controlled by gentle sponging. Some cardiac muscle adjacent to the His bundle was left attached to it. Carnoy fixation was used, and the tissue was imbedded in paraffin. Sections were cut parallel to the long axis of the Purkinje fibers and at a thickness of 25μ . These sections were then stained with hematoxylin and eosin.

Figure 1 is a low-power drawing of a section made from the left limb of the bundle of His. Comparison of the vascular supply can be made with that of the cardiac muscle which has been left attached. It can be easily made out in this section that the Purkinje fibers are much more scantily supplied with blood vessels than is the adjacent heart muscle. In fact, one of the most striking differences is the degree of vascularity. It can also

be made out that the precapillary vessels are confined, in the main, to the tissue between the Purkinje fibers, the capillaries being arranged around their periphery.

Figures 2A and 2B are high-power drawings of small areas of the Purkinje fibers (2A) and heart muscle (2B) shown in figure 1. By comparing them, one can see how scanty is the vascular supply of the Purkinje fibers in contrast to the abundant supply of the heart muscle.

The question at once arises: Is the comparative vascularity between heart muscle and the Purkinje fibers, as shown by the above illustrations, representative of the structure as a whole? A study of the right limb of the bundle reveals substantially the same degree of vascularity as is found in the left limb. Sections from the main bundle show essentially the same vascular arrangement as in the limbs, with a slight vascular increase.

Lydia DeWitt, arguing that Purkinje fibers are syncytial in character, states that no blood vessels or nerves seem to penetrate them. In not a single instance do our specimens show blood vessels penetrating the Purkinje fibers in the main bundle. In the lower portions of the limbs and in the moderator bands, where the fibers increase in size, our specimens show them pierced by blood vessels.

Meigs, in a study of the capillaries of the human heart, says: "The presence of the large capillaries and the penetration of the muscular fibers by the capillaries indicate a provision for the blood supply of the heart even more bountiful than that of the other organs." He studied both injected and uninjected specimens and showed illustrations proving this contention.

As the vascular supply of the Purkinje fibers is very scanty in comparison to heart muscle, it is probable that they do not represent tissue specialized for the same type of contractility as heart muscle.

We wish to express our thanks to Dr. E. R. Clark for his assistance during the progress of this work. We wish further to acknowledge the aid of Mr. G. T. Kline, who made the final drawings for the illustrations.

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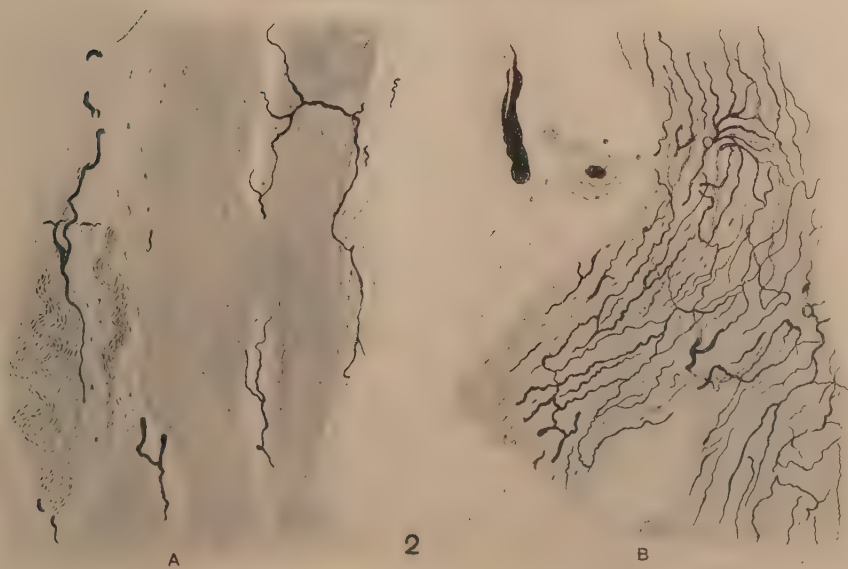
PLATE 1

EXPLANATION OF FIGURES

All drawings were made with the camera lucida set at table level.

1 Drawing of a section from the left limb of the bundle of His of the injected calf's heart. Some cardiac muscle adjacent to the bundle of His has been left attached. The sections were cut parallel to the long axis of the Purkinje fibers and at a thickness of 25 μ . They were stained with hematoxylin and eosin. $\times 72$.

2 Figures 2A and 2B are high-power drawings of small areas of the Purkinje fibers (2A) and heart muscle (2B) shown in figure 1. $\times 300$.



Resumen por W. H. Lewis, por el autor C. S. Beek.

La distribución relativa de los clasmotocitos en los diversos órganos del embrión de pollo de siete días.

En todas aquellas estructuras del embrión de pollo de siete días que contienen tejido reticular laxo existen numerosos clasmotocitos. Su número parece variar con la cantidad de este mesenquima laxo. Abundan especialmente inmediatamente debajo de la epidermis, del revestimiento endodérmico del canal alimenticio y del revestimiento mesotelial de las cavidades pleural y peritoneal. Existen muchos entre las fibras esqueléticas musculares y las fibras lisas, siendo raros en el músculo cardíaco. Abundan también en el mesenquima laxo de los pulmones y la córnea, alantoides y amnios; son raros en el mesonefros, metanefros y el bazo, faltando en la esclerótica, hueso, cartílago, retina y lóbulos ópticos. El autor no encontró ninguno en el hígado.

En algunas partes del cerebro, aun cuando en este órgano se supone que no existe mesenquima laxo, son abundantes los clasmotocitos, como ocurre en la corteza cerebral y en el tálamo. Son también numerosos en los plexos coroideos. Su distribución fué determinada mediante el exámen de frotos vivos teñidos con rojo neutro. Los granos y vacuolas se tiñen rápidamente con el rojo neutro, comunicando al clasmotocito un color rojo patente, que contrasta con el de los otros tipos de células. Esta coloración de las vacuolas grandes tan abundantes en el clasmotocito permite diferenciarlos fácilmente de todos los demás tipos en los frotos de células vivas.

THE RELATIVE DISTRIBUTION OF CLASMATOCYTES IN THE VARIOUS ORGANS OF THE SEVEN- DAY-CHICK EMBRYO

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The type of cell known as the clasmatocyte is classified under the general group of phagocytic cells to which Metchnikoff¹ gave the term *macrophage*. These cells have received various designations: for example, *clasmatocytes* by Ranvier,² *ruhende Wanderzellen* (Clasmatocyten) by Maximow,³ *cellules rhagiocrines* by Renaut,⁴ and *macrophages* by Evans and Scott.⁵ To this group also belong the macrophages which inhabit the serous cavities of the body.

The method employed in determining the relative distribution of clasmatocytes consisted of an examination of spreads of living tissue from seven-day-chick embryos which had been exposed to a solution of neutral red. Pieces of tissue, approximately a millimeter or less in each dimension, were dissected from various organs under the binocular microscope and freed entirely from the surrounding tissues. The pieces were then immersed for several minutes in a mixture of neutral red and Locke's solution in a concentration of 1:20,000, mounted, and examined immediately as living spreads.

¹ Metchnikoff, Elias, 1892. *Leçons sur la pathologie comp. de l'inflammation*. Paris.

² Ranvier, L. 1899. Des clasmatocytes. *Archiv. d'Anat., Micr.*, T. 3, pp. 123-139.

³ Maximow, A., 1906. Ueber die Zellformen des lockeren Bindegewebes. *Archiv. f. micr. Anat.*, Bd. 67, S. 680-757.

⁴ Renaut, J., 1907. Les cellules connectives rhagiocrines. *Archiv. d'Anat., Micr.*, T. 9, pp. 495-606.

⁵ Evans, H. McL., and K. J. Scott, 1921. On the differential reaction to vital dyes exhibited by the two great groups of connective-tissue cells. *Contributions to Embryology*, Carnegie Inst. Wash., Pub. 273.

The clasmatocyte of the chick, as seen in tissue treated by this method, is a relatively large cell, varying from about 10 to 20 μ in diameter. Numerous processes or pseudopodia extend from the cell-body, and these vary in number and size according to the structures surrounding the cell. In a fluid medium the clasmatocyte may be perfectly ovoid in shape; when lying between muscle-fibers, it usually possesses two processes which extend between the fibers. When the clasmatocyte is relieved of the stress exerted by the surrounding structures, as when it flows from its position between two muscle-fibers, its processes are retracted and it becomes ovoid in shape. In other places the processes of the cell are variable in number and extend irregularly from the cell-body. The cytoplasm is always relatively abundant and contains vacuoles which stain with neutral red. These vacuoles form one of the most conspicuous morphological features of the clasmatocyte. When treated with neutral red they become markedly colored; when examined unstained under the microscope they stand out prominently and somewhat resemble globules of fat. In tissue that has been run through alcohol in the process of staining, the globules are dissolved, as a result of which it is almost impossible to recognize the clasmatocyte in ordinary sections.

The clasmatocyte is, as a rule, mononuclear. The nucleus is eccentric in position and in the living cell remains unstained. It is not very uncommon, however, especially in the submucosa of the proventriculus where it was particularly observed, to find a clasmatocyte containing two nuclei. In this location the cell shows the greatest variation in morphology; the cytoplasm is usually packed with vacuoles, there may be one or two nuclei, and the processes of the cell assume irregular contours. Here, also, the cells have been observed in process of division. In most clasmatocytes that are flattened out in tissue cultures, a centrosphere, or an area free from vacuoles and fat globules, can be seen to occupy the center of the cell. The centrosphere is comparatively large and finely granular and in its center, in fixed material, is seen often a single or double centriole. The periphery is continued into the surrounding cytoplasm and

with certain fixations distinct radiations can be seen extending from the centrosphere into the cytoplasmic framework. The nucleus is always crowded off to one side of the cell and may be separated from the centrosphere by vacuoles.

In all those structures of the embryo in which mesenchyme exists, especially if it exist in a fairly loose or reticular form, clasmatoocytes are abundant. This loose or reticular mesenchyme is present in the relatively vast areas lying directly beneath the epidermis, beneath the entoderm of the entire alimentary tract, and beneath the mesothelial lining of the pleural and peritoneal cavities. A bit of tissue taken from any of these areas, it matters not whether from the subcutaneous area, the submucosa, the subserosa, the parietal wall, or the supporting mesentery, contains clasmatoocytes in the greatest abundance. This condition is common in the spreads that have been mounted with considerable fluid and simply shows the ease with which the clasmatoocyte can be removed from its position. As the differentiation of mesenchyme proceeds to the development of specialized structures, the number of clasmatoocytes progressively decreases. This is shown strikingly in the successive stages of bone formation, the reticular areas of mesenchyme containing numerous clasmatoocytes, while the contiguous and more highly differentiated areas of cartilage and bone are entirely devoid of them. This condition is shown also in the sclerotic coat of the eye, where the mesenchyme destined to form this structure has become, at the age of seven days, semicartilaginous in character and is entirely free from clasmatoocytes.

The relationship between the degree of specialization of mesenchyme and the number of contained clasmatoocytes is also shown strikingly in the musculature of the seven-day-chick embryo. At this age the musculature markedly resembles the soft, semifluid mesenchyme from which it has arisen, and one might expect to find numerous clasmatoocytes in such structures. Both the skeletal and the non-striated varieties of muscle-fibers are surrounded by abundant mesenchyme in which clasmatoocytes are very numerous. In the case of the heart, this specialization of structure has advanced to a much higher degree than that

of the other two varieties of muscle. The anastomosing muscular trabeculae make up by far the greater mass of the heart substance and the mesenchyme between the trabeculae has become specialized connective tissue of a functional character. To demonstrate the presence of clasmatocytes in the heart, either in the epicardium, endocardium, or myocardium, may require careful examination of numerous living spreads, but an occasional clasmatocyte can be found, usually more readily in the epicardium and the endocardium than in the myocardium, where they are quite rare. The same condition that exists in the heart is found also in the large vessels connected with it.

In the mesodermal layer of both the inner and the outer walls of the allantois, clasmatocytes are abundant, in numbers approximating those found in the loose or reticular mesenchyme occurring in the subcutaneous tissue and in the submucosa of the gut. The same is true of the amnion, where smooth muscle exists in a relatively loose or reticular form. Examination of the yolk sac does not reveal their presence. This is rather to be expected, in view of its histological structure; the yolk sac is essentially an entodermal structure and the picture under the microscope is greatly blurred by the presence of large glandular cells containing much fat.

The lung, embryologically, is an outgrowth from the post-branchial pharynx and at this age (seven days' incubation) consists essentially of three or four entobronchi embedded in an extensive, loose mesenchymal matrix. Clasmatocytes are almost as numerous in this loose mesenchyme of the lung as in the mesenchyme of the subcutaneous area.

The presence or absence of clasmatocytes in the structures of the eye corresponds again to the presence or the absence of loose reticular mesenchyme. At this age the mesenchyme of the sclerotic coat has become highly differentiated and contains no clasmatocytes. The choroid coat is supposed to be a differentiation of the mesenchyme surrounding the optic cup. Under the microscope it appears to be made up only of highly differentiated pigmented cells. The loose reticular mesenchyme has disappeared from this structure and clasmatocytes are correspondingly

absent. The cornea develops from two sources: the external epithelium is derived from ectoderm, the cornea propria from the surrounding mesenchyme. This mesenchyme begins to penetrate the substance of the cornea propria at the seventh day and forms the so-called corpuscles of the cornea. Clasmatoocytes are abundant in the cornea and can be found upon examination of both the inner and the outer surfaces of the structure.

The mesonephros is the principal organ of excretion during the embryonic life of the chick, and at the end of seven days of incubation it forms the greater mass of kidney substance. In conjunction with the well-developed mesonephros and Wolffian duct, there has already developed nephrogenous tissue of the metanephros with its diminutive ureter. As the development of these structures takes place in a mass of mesenchyme, furnishing, as it does, both the capsule and the connective-tissue elements of the kidney, it is not surprising to find an occasional clasmatoocyte in the connective-tissue elements lying between glomeruli or renal tubules. The number of clasmatoocytes present in mesonephros or metanephros is small. They are relatively more abundant in the walls of the Wolffian duct and the ureter than in the kidney substance.

In the spleen great numbers of cells take up neutral red. These cells do not possess the characteristic pseudopodia and the vacuoles of the clasmatoocyte. They are more regular in outline and seem to be of a more fixed nature. They are probably reticular cells. The clasmatoocyte undoubtedly can be demonstrated in the spleen, but careful search is required, as in this organ it does not constitute a conspicuous type of cell.

Clasmatoocytes were not found in the liver. This organ is remarkable in possessing no mesenchyme in the embryonic stages after the fourth day (Minot⁶). With the absence of mesenchyme it is not surprising to find an absence of clasmatoocytes in this organ. The endothelial type of macrophage is represented here by the Kupffer cells, which can be demonstrated in spreads treated with neutral red.

⁶ Minot, C. S., 1900. On a hitherto unrecognized form of blood circulation without capillaries in the organs of vertebrata. *Proc. Boston Soc. Nat. Hist.*, vol. 29.

The presence or absence of clasmatocytes in every structure so far mentioned may be accounted for adequately by the presence or absence of loose reticular mesenchyme existing in that organ. Examination of the nervous tissue shows that the brain and the spinal cord do not parallel this general principle, because clasmatocytes are not uniformly distributed throughout the nerve substance; they are, in fact, absent from the optic lobes of the brain and from the retina. The cortical hemispheres contain clasmatocytes and throughout the corpus striatum they are relatively abundant. In the regions of the choroid plexus, in the telencephalon medium, and in the lower portions of the thalamus, clasmatocytes are quite numerous.

Mention should be made of the existence of a type of cell to which no reference could be found in the literature. These cells vary considerably in size, being approximately $2\frac{1}{2}$ to 4μ in diameter and irregularly spherical or ovoid in shape. The nucleus is not a conspicuous structure. The cytoplasm contains granules of variable size which take up neutral red and the cytoplasm itself becomes more or less diffusely colored. These cells are of widespread distribution, occurring in greatest numbers in the brain, retina, and kidney, but their presence can be demonstrated in almost every tissue of the chick.

SUMMARY

1. The clasmatocyte is present in great numbers in all those structures of the seven-day-chick embryo that contain loose reticular mesenchyme.

2. The number of clasmatocytes present in mesenchymal structures seems to vary with the amount of loose reticular mesenchyme.

3. The brain, excluding the optic lobes and retina, is the only structure supposedly containing no loose reticular mesenchyme in which clasmatocytes are present.

Resumen por el autor, F. A. McJunkin.

Teñido de la peroxidasa con la benzidina en cortes de parafina
de tejidos humanos

Sexta comunicación sobre los estudios de los leucocitos mononucleares de la sangre.

Las secciones en parafina pueden utilizarse para el teñido de la peroxidasa cuando se observa la siguiente técnica en su preparación: Inclusion—1) Piezas de tejido de un milímetro de espesor, fijadas en formol se transportan á una solución de acetona al 70 por ciento en la que permanecerán durante una hora; 2) Acetona, 30 minutos; 3) Benzol, 20 minutos; 4) Parafina, 20 minutos. Teñido—1) Los cortes de 3.5 á 5 micras de espesor se pegan al portaobjetos con albúmina glicerizada dejándolos secar durante la noche á la temperatura de la habitación; 2) Se disuelve la parafina mediante el benzol (20 segundos) y la acetona (10 segundos); 3) Se sumergen los portaobjetos en agua durante unos segundos, se quita el exceso de agua y se tiñen durante 5 minutos en una solución de benzidina (25 centímetros cúbicos de alcohol metílico al 80 por ciento, 100 miligramos de benzidina y, 2 gotas de peróxido de hidrógeno), diluída en el momento de usarla en una ó dos partes de agua destilada; 4) Se lavan los portaobjetos en agua durante 5 minutos y se tiñe con hematoxilina de Harris durante 2 minutos; 5) Nuevo lavado en agua durante 1 minuto y se tiñe con eosina (0.7 por ciento) durante 20 segundos; 6) Se deshidrata con alcohol de 95° (30 segundos) y alcohol absoluto (5 segundos); 7) Xilol; 8) Montaje en bálsamo.

Las células que dan la reacción (color pardo) son las neutrofílicas y los leucocitos y mielocitos eosinofílicos, las células mononucleares de la pulpa esplénica y los sinusoides del hígado, y los leucocitos mononucleares de los vasos sanguíneos.

PEROXYDASE STAINING WITH BENZIDIN IN PARAFFIN SECTIONS OF HUMAN TISSUE

SIXTH REPORT OF STUDIES ON THE MONONUCLEAR LEUKOCYTES OF THE BLOOD

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FOUR FIGURES

A practical method for the peroxydase staining of histologic sections originated with Schultze,¹ although it had previously been observed that certain tissues and tissue products developed color compounds in dilute solutions of naphthols and phenols. By the oxydase reaction of Schultze, frozen sections of formalin-fixed tissue are treated with an aqueous solution of alphanaphthol followed by an aqueous solution of dimethyl-p-phenylene-diamine, with the production of a blue color in myeloblastic cells and no color change in cells of the lymphocyte series. Since these two chemicals in the test-tube unite in the presence of an oxydizing substance to form indophenol blue, the supposition is that the reacting granules in the leukocytes perform the function of an oxydizing agent. The outcome of this conception has been that the reactions in the numerous subsequent methods are spoken of as peroxydase or oxydase, according to whether an additional oxydizing substance such as hydrogen peroxide is employed or not.

Guaiac, alpha-naphthol, benzidin, resorcin, hydroquinon, and pyrogallol are some of the more common compounds known to yield color reactions with tissue. Benzidin unites with numerous amines and phenols to form dyes of the azo group. The exact way in which the cytoplasm of certain cells acts on benzidin to form the brown color elicited is not known and the

use of the terms peroxydase and oxydase may ultimately prove inappropriate. These terms have, however, come into general use and the reacting substance in its behavior to elevated temperatures, solubility in alcohol, and in other ways resembles known enzymes. Of the substances used for the reaction, benzidin yields the most precise and intense staining and the colored compound developed permits the application of the common contrast stains.

That the myeloblastic cells with eosinophilic and neutrophilic granules contain an oxydizing enzyme, while the cells of the lymphocyte series do not, has quite generally been accepted. Few statements in regard to the behavior to benzidin, alpha-naphthol and other substances of non-lymphocytic mononuclear leukocytes and of endothelial cells of lymph and blood vessels have been made. Graham² observed areas of reacting cytoplasm in endothelial cells lining capillaries especially in the liver, and suggested that such reactions are due to particles of disintegrated myeloblastic cells ingested by the lining endothelium. The author,^{3,4} working with peripheral blood, found that many of the mononuclear leukocytes which ingest carbon react to benzidin. The intravascular ingestion of carbon by these leukocytes as well as the phagocytosis of carbon particles in vitro by them is accomplished by adding sodium citrate to a concentration sufficient to inhibit the phagocytic activity of the polymorphonuclear neutrophiles. The benzidin reacting cells of normal peripheral blood are the neutrophiles, eosinophiles and many of those mononuclear cells which are commonly spoken of as 'large mononuclear and transitional' leukocytes.

To determine the reaction of various tissues to benzidin for comparison with cells in blood smears giving the peroxydase reaction, frozen sections of various organs were treated with benzidin but with unsatisfactory results. In the methods recommended only frozen sections or teased bits of formalin-fixed tissue have been available, since the reactive property is destroyed by the usual paraffin or celloidin method of imbedding. In 1918 the author⁵ published a method for imbedding tissue in soap in which the granules are preserved. Although the soap

sections have proved superior in some respects to frozen ones, they are inferior to those obtained by paraffin imbedding. The purpose of this paper is to give a method for the preparation of paraffin sections with preservation of peroxydase-reacting granules and to describe the results obtained by the method on human tissue.

Method

The method which preserves the reacting substance in certain mononuclear and marrow types of cells is as follows:

Imbedding. 1. Bits of formalin-fixed tissue 1 mm. thick are placed in 70 per cent acetone for one hour.

2. Acetone, 30 minutes.

3. Benzol, 20 minutes.

4. Paraffin, 20 minutes.

Staining. 1. Sections 3.5 to 5 μ in thickness, cut in the usual way, are attached to slides with albumin-fixative and allowed to dry overnight at room temperature.

2. Remove paraffin with benzol (20 seconds) and acetone (10 seconds).

3. Plunge the slide into water for a few seconds, remove the excess of water and apply for 5 minutes a benzidin solution (25 cc. of 80 per cent pure methyl alcohol, 100 mgm. benzidin and 2 drops hydrogen peroxide) diluted at the time of placing it on the preparation with one or two parts of distilled water. Two parts give less intense staining. The alcoholic benzidin solution is permanent.

4. Plunge into water for 5 minutes and stain with hematoxylin (Harris) for 2 minutes.

5. Plunge into water for 1 minute and stain with eosin (0.1 per cent) for 20 seconds.

6. Dehydrate with 95 per cent alcohol (30 seconds) and absolute alcohol (5 seconds).

7. Xylol.

8. Mount in balsam.

In order to determine that none of the peroxydase reacting property of the cytoplasm is destroyed in the embedding process,

a second method is available in which an initial treatment with benzidin is performed. It has no advantages over the one given above and has a tendency to color cells diffusely. The method is as follows:

Method

1. Fresh tissue is fixed in 10 per cent formalin, cut into very thin bits less than 1 mm. thick, and washed in running water for 30 minutes.

2. Two hours in a benzidin solution prepared immediately before placing the tissue in it by mixing 7 parts of acetone with 2 parts of distilled water and 1 part of hydrogen peroxide and adding 2 mgm. of benzidin to each cc. of mixture.

3. Acetone, 30 minutes.

4. Benzol, 20 minutes.

5. Paraffin, 20 minutes.

Paraffin sections are cut in the usual way and stained with hematoxylin and eosin as in the first method.

The reacting cytoplasm which is arranged in the form of more or less definite granules is colored yellow to brown. The initial color formed is blue, but this soon changes to brown. In sections the cytoplasm overlying the nucleus tends to obscure the nuclear staining, but the chromatin of the nucleus appears not to react.

It is sometimes desirable to color by the Berlin-blue reaction hemosiderin which may be present. This is done by placing the benzidin-treated sections for 5 minutes in equal parts 1 per cent potassium ferrocyanide and decinormal hydrochloric acid, washing them in the dilute acid followed by water, and proceeding with the hematoxylin-eosin stain in the usual way. Hemosiderin is colored blue, while any brown color brought out by the peroxydase reaction is unchanged by the test.

In unfixed tissue the reacting substance disappears soon after death, and the rate of its disappearance cannot be measured entirely by the time which elapses. After the tissue has become completely fixed in formalin the intensity of the peroxydase staining slowly decreases.

TISSUE REACTIONS IN BENZIDIN-PARAFFIN SECTIONS

A control section of all tissues from which conclusions are to be drawn must be made by staining in the usual way a section which has not been treated with benzidin. This is especially important in the case of the liver, spleen, and lymph nodes. The commonest yellowish or brownish pigments are the hemosiderins. An iron reaction may be performed on the benzidin preparation as indicated under the directions for performing the stain.

The action of the benzidin solution is specific in the sense that a few varieties of cells regularly react, while the other cells and tissues show no reaction. Briefly, in human tissue no cells are colored by the benzidin except, *a*) myeloblastic cells with neutrophilic and eosinophilic granules; *b*) certain mononuclear cells which are most abundant in the spleen, and, *c*) mononuclear leukocytes which may be found in the blood vessels of any tissues of the body, but which are seen best in the sinusoids of the liver and the sinuses of the spleen. The reaction of the myeloblastic cells is very intense and requires little discussion.

Spleen. A large number of mononuclear cells giving a peroxylase reaction are present. Not only is the number much greater than is found in any other organ, but the benzidin-colored cells are sufficiently numerous to make them a conspicuous feature of the microscopic appearance of the spleen. With low magnification they are seen to occur in the splenic pulp between the lymph nodules, which themselves are quite free from reacting cells. The endothelial cells of the characteristic venules have not been demonstrated to react. It may be stated that the reacting mononuclear cells correspond in location and morphology to those usually termed 'splenic or pulp' cells. They vary in size from slightly larger than neutrophils to two or three times that size. The vesicular nucleus is usually indented or saddle-shaped, but sometimes round. In the distinct cytoplasm occurs the brown granulation and sometimes hemosiderin granules, or ingested carbon particles, red blood corpuscles, lymphocytes, or neutrophils are present. Material ingested by phagocytosis does not alter the peroxylase staining of the cytoplasm about it.

The general cell outline tends to be regular, but some cells present projections in one direction or another. The supporting reticular cells do not react. The endothelium of the larger blood vessels does not color with the benzidin.

It is difficult to determine the relation of the reacting cells to other cells of the blood-containing pulp spaces and especially to cells which may or may not line these spaces. Excluding the reticular cells of the pulp and those lining definite blood sinuses, the reacting cells may constitute a majority of those present in the pulp. Mitoses in the cells colored by the benzidin have been observed. The character of both the nucleus and cytoplasm as well as the tendency to ingest non-bacterial material serves to distinguish these cells from myelocytes. The chief reason that the possibility has not been entertained of the mononuclear leukocytes of the blood and of these reacting cells in the lumina of vessels and in the spleen being of marrow origin is that they lack the regularity in size and shape of myelocytes. Congestion and the collection of neutrophils in the vessels obscure the normal histologic picture.

In the routine examination of sections of spleen cut through the capsular surface occasionally the mesothelial peritoneal covering is found to consist of cuboidal or columnar cells. These cells do not react to benzidin.

Liver. In this organ is found one of the most favorable tissues for the study of cells which line capillary blood vessels. Here it is easier to define accurately the lining endothelial cells than it is in the spleen. Within the sinusoids are neutrophils and a variable number of free cells of the large mononuclear leukocyte type which react with the benzidin. In a few of the endothelial cells lining the sinusoids and the Kupffer cells there are peroxidase granules diffusely distributed. In a somewhat greater number there are spherical focal areas of reacting cytoplasm located usually near one end of the nucleus. The reacting spherules are not surrounded by a colorless zone or halo and do not have the appearance of particles ingested by phagocytosis.

Lungs, kidney, pancreas, adrenal, and heart are other organs which have been examined from a small number of autopsies.

Extravascular neutrophilic and non-lymphocytic mononuclear leukocytes are scanty in these situations when acute and chronic inflammatory lesions are absent. Neutrophiles and an occasional large mononuclear leukocyte with positive benzidin reaction occur within the blood vessels. The cells lining the capillaries of these organs do not react. The spleen, liver, and lymph nodes have been examined from about fifty autopsies.

Lymph nodes. Owing to the divergent views of the origin of blood-mononuclear leukocytes and tissue wandering cells, especial interest attaches to the reaction of the cells present in the sinuses and in the germinal centers of lymph nodes. Within the sinuses of a non-inflamed lymph gland there are a few neutrophiles, but usually phagocytic and non-phagocytic large mononuclear cells occur and they are often abundant. The mononuclear ones do not react to benzidin. Numerous glands obtained at autopsy have failed to show a trace of reaction in the large mononuclear cells. The phagocytic cells may contain neutrophiles, lymphocytes, blood corpuscles, carbon particles, or fat droplets, but the cytoplasm about the ingested particles like that of the non-phagocytic ones does not react. The endothelial cells lining the sinuses and the reticular cells extending across them are likewise devoid of reacting granules. The cells of the lymph nodules and the germinal centers do not react. In a few instances a limited number of reacting mononuclear cells have been present in the sinuses. The large mononuclear cells in and near the lymph sinuses which often contain ingested corpuscles, neutrophiles, or lymphocytes are larger and possess a larger amount of eosin-staining cytoplasm than is seen in the peroxydase-staining mononuclear cells of the spleen.

DISCUSSION AND SUMMARY

The peroxydase reaction has usually been interpreted as a means for the separation of the reacting myeloblastic cells from the non-reacting cells of the lymphocyte series. In blood smears even a casual examination shows that many of the leukocytes spoken of as 'transitional and large mononuclear' react positively to benzidin. The origin of such mononuclear leukocytes

and of tissue wandering cells has been and is now a matter of controversy that is not likely to be settled by proof of a single kind. Observers agree that these wandering cells and the endothelial cells lining the blood capillaries of some organs and the lymph channels in certain locations are both phagocytic, especially for non-bacterial substances such as fat, disintegrating cells, and blood corpuscles. If the wandering cells are considered derivatives of such attached phagocytic cells, it is at least unnecessary to assume that they acquire a new function when they become free; so far as phagocytic behavior is concerned, the large mononuclear leukocyte group of the blood and these attached cells are alike.

The endothelial cells of organs with an extensive capillary network undergo mitosis in locations where the capillary does not appear to be lengthening and become free in the lumen. From this observation and the close resemblance in morphology especially after the ingestion of carbon the conclusion drawn by the writer⁶ was that the large mononuclear and transitional cells of the blood arise in this way. The observations from which this conclusion was drawn were made almost exclusively on the liver where endothelium can be studied to the best advantage. The benzidin preparations show that a few of the endothelial cells of the liver react positively, but the number of peroxydase staining mononuclear cells is so great in the spleen that it seems likely that this is an important source of mononuclear blood cells with benzidin granules.

The absence of peroxydase-staining cells in the lymph glands is considered significant. In blood smears to which a peroxydase stain has been applied there are present cells larger and with more cytoplasm than lymphocytes which have nuclei more vesicular than lymphocytes and which do not react. In normal human blood such non-reacting cells constitute a minority of the leukocytes of the 'large mononuclear-transitional' type. Are such cells derived from the lymph sinuses? Summarizing previous work, McClure⁷ has called attention to the conclusiveness of the proof of the local origin during embryonic development of vascular endothelium from mesenchyme. The endothelium

of the lymph vessels may arise in parts of the embryo where there is mesenchyme and with no connection with blood vessels. The blood spaces of the splenic pulp develop in the embryo in close association with the blood vessels. That the cells differentiating in this location in connection with blood vessels are in the adult essentially different from endothelium of the lymphatics is indicated by the response to benzidin.

CONCLUSIONS

1. Thin bits of formalin-fixed tissue can be passed through acetone and benzol and embedded in paraffin with preservation in all cells having the property of giving the reaction of the substance on which peroxylase staining is dependent.

2. The cells which react positively to the benzidin are, *a*) myeloblastic cells with neutrophilic and eosinophilic granulations; *b*) mononuclear cells present in the splenic pulp and focal areas in the cytoplasm of some of the endothelial cells of the liver sinusoids, and, *c*) large mononucleated leukocytes within the blood vessels.

3. The mononuclear cells of the splenic pulp which give the reaction are present in the spleen of all individuals, and in morphology they are similar to the peroxylase-staining mononuclear leukocytes within the blood vessels. The irregularity in size and shape of the reacting splenic cells indicates that they are not myelocytes. However, the data obtained by peroxylase staining do not exclude the possibility of some of these cells being myelocytes. The relation of these cells to the vascular endothelium in the location where they occur is not clear.

4. The large mononuclear cells regularly present in the lymph sinuses of lymph glands which become phagocytic and present such a conspicuous feature of certain mild lymph node inflammations do not react to benzidin. The positive identification in other tissues of the cells found in the lymph-node sinuses probably awaits the development of a technic characterizing them.

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PLATE 1

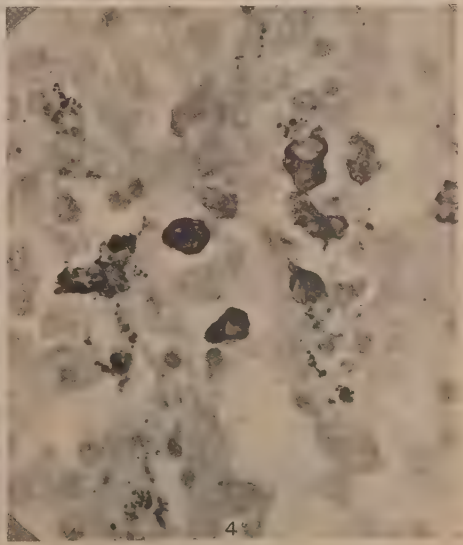
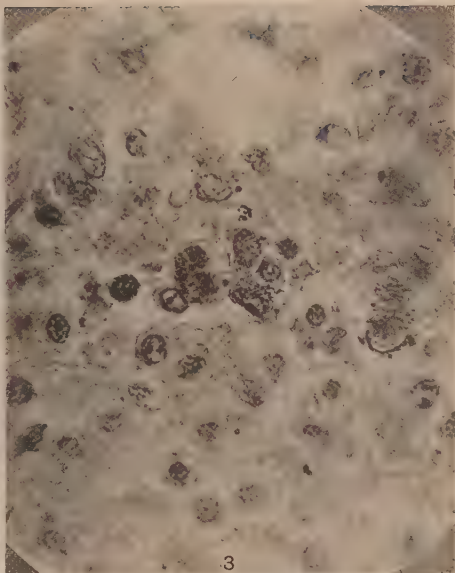
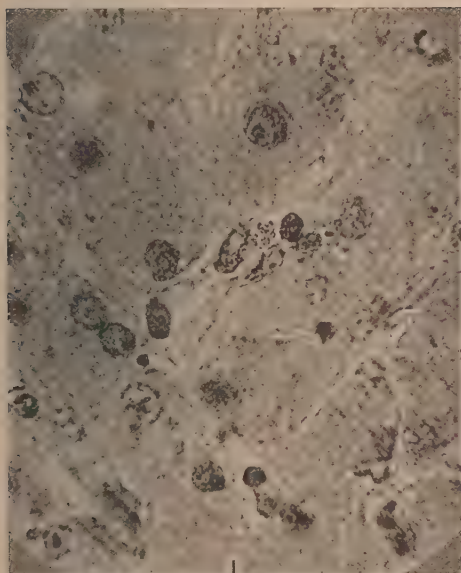
EXPLANATION OF FIGURES

1 Liver showing an endothelial cell of a sinusoid with cytoplasm containing numerous peroxydase granules. None can be distinguished in the cell on the opposite side of the same sinusoid.

2 Liver with Kupffer cell which contains two circumscribed spherules of reacting cytoplasm.

3 Spleen with three peroxydase-staining mononuclear cells at center of field. Two of them have indented nuclei. Cytoplasm of lymphocytes is homogeneous.

4 Spleen showing mononuclear cells and a neutrophile with peroxydase granules.



Resumen por el autor, A. I. Salazar.

Sobre la forma de la degeneración de los folículos anovulares de Regaud y de otros restos que provienen de los cordones ovígenos del ovario de la coneja.

La aplicación del método tanoférrico al ovario de la coneja ha permitido al autor por vez primera el poner en evidencia el proceso de degeneración de los llamados folículos anovulares de Regaud y las formaciones análogas. Esta degeneración, que el autor llama "atresia hidrópica" consiste en la acumulación en los intersticios de las células del residuo de una sustancia que se tiñe en negro oscuro mediante el método mencionado anteriormente. Esta sustancia resiste tenazmente á la decoloración con el ferrocianuro de Weigert.

El magma hidrópico crece progresivamente, disociando al residuo, que finalmente se disgrega. Los restos del magma y unas cuantas células, que han escapado á la destrucción, se extienden en el tejido conectivo. El magma hidrópico parece resultar de la alteración atrésica de una delgada capa del líquido que existe normalmente entre las células del residuo. La membrana propia del residuo se hipertrofia á veces, transformándose en un cordón tortuoso, teñido intensamente en negro; este cordón tortuoso á menudo permanece aislado en el tejido conectivo después de la disgregación del residuo. La hipertrofia de la membrana propia es enteramente análoga á la membrana atrésica de Slayjansky. A veces se encuentra asociada con el proceso hidrópico una acción disociativa del tejido conectivo, siempre muy ligera; este hecho es interesante, porque esta acción disociativa, en mucho menor grado, reproduce un proceso característico de la atresia conectiva fetal de Winiwarter.

SUR LA FORME DE DÉGÉNÉRESCENCE DES FOLLICULES ANOVULAIRES DE REGAUD ET D'AUTRES RELIQUATS PROVENANT DES CORDONS OVIGÈNES DE L'OVAIRE DE LA LAPINE

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DIX FIGURES

La méthode tannoferrique¹ met nettement en relief la forme de dégénérescence des follicules anovulaires de Regaud et d'autres reliquats épithéliaux dérivés des cordons ovigènes. Le processus involutif de ces formations n'est pas encore connu: tout récemment Gérard² écrivait: "Que deviennent ces nodules épithéliaux? Nous n'en avons jamais vu dégénérer." Les formations en question dégénèrent par un processus qu'on peut appeler "hydropisie tannophile." Ce processus consiste dans l'accumulation d'une substance liquide ou pâteuse dont le coagulum de fixation prend une coloration intense d'un noir encre de Chine après fixation dans le liquide de Bouin et traitement par le tannin-fer (fig. 1, 2 et 3). Cette substance est très fortement tannophile, car, non seulement elle se colore très intensivement, mais encore résiste avec ténacité à la décoloration par le ferricyanure de Weigert. Quand cette différenciation est poussée très loin, jusqu'à décoloration de la plupart des autres formations tannophiles, la substance hydropique résiste encore; sa tannophilie égale ou excède celle des magma atrétiques. Cette substance hydropique s'accumule de plus en plus dans les interstices entre les cellules (fig. 1), atteint des proportions considérables (fig. 2 et 3) et finit par dissocier le reliquat dont les cellules

¹ A. L. Salazar. Méthode de coloration tanno-ferrique. *Compt. Rend. Soc. de Biol.*, T. 83, p. 1655.

² Gérard. Contribution à l'étude de l'ovaire des Mammifères. *Arch. de Biol.*, 30, 2^e part, p. 371.

périssent noyées dans le magma ou deviennent libres. Ces cellules libres se répandent dans le strome conjonctif; parfois un certain nombre de cellules s'entourent d'une membrane propre et reconstituent un nodule plus petit (fig. 5 et 9). Ce dernier fait n'est pas constant. Nous ignorons la destinée de ces cellules. Dans certains cas la membrane propre du reliquat, que la méthode tanno-ferrique dessine nettement en noir, s'hypertrophie à l'un des pôles du reliquat, devient flexueuse et se colore intensivement en noir (fig. 4 et 5); ce fait est entièrement comparable à l'hypertrophie de la membrane de Slavjansky dans la période agénique de l'atrésie (période post-chromatolytique). La forme du cordon, sa tannophilie, etc., sont absolument identiques; il n'y a qu'une différence de dimensions, plus réduites dans les reliquats.

Dans certains cas le processus de dégénérescence est plus complexe. Outre l'hydropisie tannophile, il existe une activité dissociante du tissu conjonctif, qui rappelle l'atrésie conjonctive de Winiwarter, mais cette activité dissociante du tissu conjonctif est ici très discrète et son rôle secondaire (fig. 4 et 7). Ce phénomène est intéressant, car il représente chez l'adulte un processus caractéristique de la vie embryonnaire de l'ovaire. Ce processus, d'un autre côté, n'est qu'une continuation

Toutes les figures ont été copiées de coupes d'ovaires fixés dans le liquide de Bouin et colorées par la méthode tanno-ferrique.

Figs. 1, 2, et 3 Reliquats épithéliaux en dégénérescence hydropique. Fig. 1, magma hydropique encore peu avancé; fig. 3, magma très avancé; fig. 2, un état intermédiaire.

Figs. 4 et 5 Deux reliquats avec membrane propre, hypertrophiée et flexueuse, analogue à la membrane de Slavjansky atrétique.

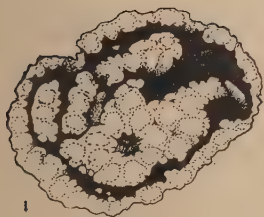
Fig. 6 Un autre aspect de reliquat en atrésie.

Fig. 7 Reliquat dissocié par l'action du tissu conjonctif.

Fig. 8 Reliquat dont le magma hydropique présente l'aspect d'un cordon flexueux.

Fig. 9 Débris provenant de l'atrésie d'un reliquat avec un petit nodule de cellules intactes.

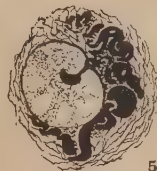
Fig. 10 Follicule de de Graaf primordial en atrésie avancée: oocyte, débris fibreux de la granulosa, membrane hypertrophiée, sont encore visibles. Cette figure est destinée à éviter la confusion avec l'atrésie hydropique des follicules anovulaires.



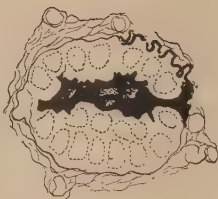
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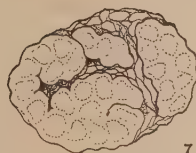
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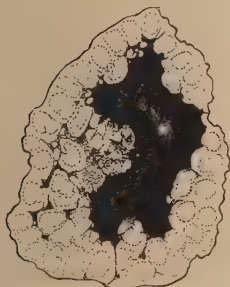
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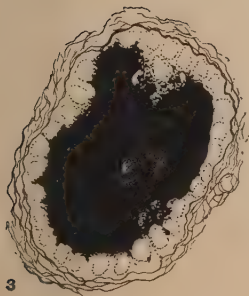
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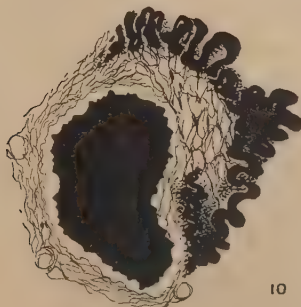
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languissante du processus normal d'amputation des cordons ovigènes.³

Le magma hydropique semble dériver de l'accumulation atrétique d'un liquide, qui normalement existe dans les interstices entre les cellules qui constituent le reliquat. Ce liquide, homologue du liquor folliculi interstitiel,⁴ se teint en gris ou gris-noir, tandis que le liquor folliculi dans les ovisacs petits et moyens se teint en noir pur.⁴

Les coagula hydropica résultants de la désagrégation des reliquats se répandent dans le strome conjonctif, où ils restent longtemps sous la forme de masses, de blocs, de cordons souvent très longs (fig. 9), parfois capricieux, tout cela coloré en noir intense par la réaction tanno-ferrique. Ces débris tannophiles disparaissent à la fin, canalisés probablement par les lymphatiques ou par les capillaires sanguins, car ceux-ci sont souvent remplis d'une substance très tannophile et abondante.

Les reliquats en pleine dégénérescence hydropique existent dans tous les types d'ovaires: ovigènes, folliculaires, atrétiques, interstitiels-cloisonnés, interstitiels-diffus.⁵ Dans les vieux ovaires (type interstitiel) le nombre des reliquats en plein processus hydropique ou encore intacts diminue, tandis que les débris tannophiles résultants des reliquats dégénérés dans les étapes antérieures (ovigène, folliculaire, atrétique) semblent devenir plus nombreux. La destruction des follicules anovulaires semble, donc, plus intense dans les ovaires plus jeunes et se ralentir et s'épuiser au fur et à mesure que l'ovaire vieillit. Nous avons trouvé des reliquats en pleine dégénérescence hydropique logés dans le centre de corps jaunes atrétiques sans vestiges de follicules. Il s'agit probablement d'un reliquat juxtaposé à un follicule, celui-ci ayant disparu plus rapidement. Cependant ces cas suggèrent l'hypothèse de la formation de corps atrétiques autour des follicules anovulaires de certaines dimensions.

³ A. L. Salazar. Sur les cordons ovigènes, etc. *Compt. Rend. Soc. de Biol.*, 1921, I, p. 235.

⁴ A. L. Salazar. Sur le follicule de de Graaf non atrésique. *Compt. Rend. Soc. de Biol.*, T. 83, p. 1658.

⁵ A. L. Salazar. Sur l'évolution de l'ovaire adulte de la Lapine. *Compt. Rend. de la Soc. de Biol.*, No. 30, T. 85.

La forme de dégénérescence, qui vient d'être sommairement décrite, rend admissible l'hypothèse d'une action endocrine appartenant au groupe des sécrétions endocrines à forme dégénérative.⁶ Parmi les variations que peut présenter le processus, celle qu'on trouve représentée dans la fig. 8 présente un intérêt spécial. Le magma hydropique a pris la forme très nette d'un cordon flexueux plus ou moins long: on dirait qu'un vermicule noir se trouve logé dans le nodule. Il existe du reste des formes de transition qui rattachent cette forme aux magma habituels. Le cordon tannophile en question est analogue à celui qu'on trouve dans les "follicules à cordon tannophile," que nous avons décrits dans l'ovaire de la Lapine.

On ne doit pas confondre les follicules anovulaires atrétiques avec l'atrésie des petits follicules de de Graaf. Dans un état avancé d'atrésie ces follicules présentent dans les coupes colorées par le tannin-fer un aspect qui ressemble d'abord aux follicules anovulaires en état avancé de dégénérescence (fig. 10).

L'absence d'hypertrophie de la thèque ne peut servir pour établir la distinction, car l'atrésie des follicules très petits n'est pas suivie de la formation de corps jaunes atrétiques. D'un autre côté, les follicules anovulaires présentent souvent une hypertrophie de la membrane propre entièrement analogue à celle que subit la membrane de Slavjansky. Cependant la distinction est presque toujours facile; les follicules véritables présentent des débris fibreux reliant le magma atrétique oocytaire à la membrane folliculaire hypertrophiée; le magma noir oocytaire est presque toujours reconnaissable, etc.

⁶ A. L. Salazar. Les corpuscules concentriques de la granulosa atrésique de la Lapine. *Compt. Rend. Soc. de Biol.*, T. 84, p. 387.

Resumen por el autor, Charles E. Buell.

Notas sobre técnica.

I. Un método para preparar embriones jóvenes montados enteros.

El embrión se fija en formol (10 por ciento) durante doce horas, lavándole en agua y tiñéndole con hematoxilina. Se destiñe en 5 por ciento ó lo por ciento de NaOH hasta que haya desaparecido todo el color azul y la preparación adquiere una tinta parda clara. Se deshidrata y se monta en bálsamo. El resultado se asemeja al obtenido mediante la impregnación argéntica.

II. Disección en parafina.

El embrión inyectado se incluye en parafina del modo ordinario. La disección se lleva á cabo en el bloque de parafina bajo el microscopio binocular, con fuerte aumento. Se disuelve la parafina en xilol, y se monta el embrión en bálsamo.

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Cornell Medical College, New York

NOTES ON TECHNIQUE

I. A METHOD OF PREPARING WHOLE MOUNTS OF EARLY EMBRYOS

II. DISSECTION IN PARAFFIN

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I. A METHOD OF PREPARING WHOLE MOUNTS OF EARLY EMBRYOS

In the preparation of total mounts of young embryos, both injected and uninjected, certain well-known difficulties are encountered. If the embryos are mounted unstained, the tissues become too transparent in the clearing reagents. Many stained preparations are so dense that injected vessels and individual structures are obscured. Staining in haematoxylin, carmine, or acid fuchsin gives excellent results in the preparation of very young blastoderms. However, in the stages beyond fifty hours of incubation, owing to the multiplicity of structures and increased density of the tissues, the study of stained embryos is rendered very difficult for the beginner in embryology.

The following technique has certain advantages in that it renders the older stages more transparent and at the same time brings the individual structures into sharp delineation. It offers a variation from the usual laboratory methods and gives a preparation that is impervious to time, acid balsam, and the bleaching effect of sunlight.

The embryo is fixed in formalin (10 per cent) for twelve hours, preferably in a flat-bottomed shallow jar with just enough of the fixative to cover the embryo. This insures a flat preparation—a decided advantage in the case of chick blastoderms. Stain the embryo for two hours in haematoxylin and allow it to decolorize in a 5 or 10 per cent solution of NaOH. The time necessary for the decolorization depends upon the density of the stain, the

strength of the alkali used, and the age of the embryo. In the case of the younger stages, the weak alkali serves best and the time for decolorizing is short, from one to two hours. With the older embryos the stronger alkali and a longer period for decolorization is necessary. When all the blue color has disappeared and the preparation has become a light yellowish-brown tint, the decolorization is complete and the optimum density has been obtained. The blood vessels stand out as brown cords retaining their cell structure. The various organs are stained different shades of brown depending upon the density of their tissues. The whole preparation is translucent and resembles effects obtained by impregnation with silver.

The embryos are washed in several changes of distilled water, dehydrated in graded alcohols, cleared in xylol, and mounted in balsam. Greatest care should be observed, as the embryos are very fragile. Glass supports for the cover-glass must be used to prevent crushing of the specimen. The balsam should be as nearly colorless as possible. Under the binocular microscope with transmitted light, the various structures are readily made out. The mounts are not affected by acid in the balsam, nor are they bleached by sunlight; in fact, they improve with age.

II. DISSECTION IN PARAFFIN

In embryological studies involving injections of the vascular tree, errors in interpretation are prone to occur because of the similarity of arteries to veins and the close interrelation of such structures. It is of great assistance to the illustrator as well as the research worker that the vessels to be demonstrated shall be isolated as far as possible from neighboring structures and that all overlying vessels be removed. In demonstrations for the class-room, it is of a decided advantage that the preparation be as little complicated as possible.

During a recent study on the origin of the pulmonary vessels in the chick, the study of whole mounts of injected embryos was difficult, in that the large cardinal veins and other structures obscured the delicate pulmonary capillaries beneath. In older

embryos this difficulty increased. I have had good results with a simple method of dissection that could be applied to other studies of embryonic circulation.

The injected chick is embedded in paraffin by the usual method and trimmed into a block with its broad surface parallel to the plane of the vessels to be demonstrated. In the case of the capillaries of the lung, this was the sagittal plane of the embryo. With gentle heat fix the block upon a glass slide. In a good light under the high power of a binocular microscope, holding a scapel lightly in the fingers of the right hand, shave off successive layers of paraffin until all the overlying vessels have been removed. A small window may be formed in this manner, exposing the desired structures and at the same time retaining the form of the embryo for localization and proportion. With a little practice and familiarity with the anatomy of the structures to be demonstrated, the result is in direct ratio to the ingenuity of the dissector. If desired, the block may be reversed upon the slide and the opposite side dissected in the same manner.

After these procedures have been carried out, the block is placed in xylol to dissolve the paraffin and mounted in balsam. In case of injections with dilute ink, the preparation is improved by staining to a light lavender tint with haematoxylin. This method eliminates a fluid medium, gives adequate fixation of the embryo during dissection, and protects the neighboring structures from injury during manipulation.

